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Acid-Base and Ionic Regulation, During and Following Emersion, in the Freshwater Bivalve, *Anodonta grandis simpsoniana* (Bivalvia: Unionidae)

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Abstract. Specimens of the boreal clam, *Anodonta grandis simpsoniana* were emersed at 10°C for 6 days and then reimmersed for 24 h. The clams lost water at a rate of 1.6% total water per day. After 144 h of emersion, water weight had declined by almost 15%, while extracellular fluid (ECF) osmolality had increased 30% to 52 mOsm kg^{-1}. Control levels were reattained after 6 h reimmersion. ECF P_{O_2} declined rapidly in the first 24 h of emersion, but remained near 20 Torr for the full 6-day exposure. After an initial rapid fall, pH declined at a slower rate, reaching 7.494 ± 0.037 (mean ± SEM) at 144 h. P_{CO_2} was elevated from 0.6 ± 0.6 to 12.4 ± 1.1 Torr after 96 h, but no further increase was noted. ECF [Ca] increased threefold to 13.1 ± 0.8 mmol l^{-1}, while [HCO_3app] rose from 5.4 ± 0.3 to a maximum of 12.9 ± 0.8 mmol l^{-1} after 144 h. ECF [Na] and [Cl] were not affected by emersion. On reimmersion, recovery was rapid, with pH, P_{O_2} and P_{CO_2} returning to control within 2 h, while [Ca] and [HCO_3app] remained elevated until 24 h after reimmersion. A 1:1 stoichiometry between [Ca] and [HCO_3app] existed throughout the emersion and reimmersion periods. In the absence of protein buffers, the fall in ECF pH was arrested by the mobilization of calcium carbonate, presumably from the shell. By 96 h emersion P_{CO_2} and P_{O_2} had stabilized, suggesting that diffusion gradients sufficient to allow limited gas exchange had been established.

Introduction

Marine intertidal bivalves experience cyclical episodes of emersion and reimmersion, and their physiological and behavioral responses have been documented (see reviews by McMahon, 1988; Shick *et al.*, 1988). In the freshwater environment, however, changes in water level and emersion events are unpredictable; their timing and duration is dependent on such factors as rainfall, temperature, and physical changes in the watershed. The responses to emersion of the freshwater bivalves that inhabit the shallower regions of such systems are less well known. As an adaptation to this stress, some species of freshwater bivalve can withstand emersion for up to a year (Hiscock, 1953).

An emersed clam is faced with opposing needs: water conservation, accomplished by valve closure, and respiration, requiring valve opening. In addition, the problems of acid-base balance, ion regulation, and excretion are all exacerbated by aerial exposure. Intertidal bivalves need only withstand approximately 12 h emersion before the next tidal inundation. Even so, many intertidal bivalves have evolved compensations, chiefly respiratory, that enable them to conserve energy during emersion. Examples include valve gaping and aerial gas exchange (Boyden, 1972; Widdows *et al.*, 1979) and the use of shell $CaCO_3$ for maintaining acid-base balance (Crenshaw and Neff, 1969; Crenshaw, 1972; Akberali *et al.*, 1977; Booth and Mangum, 1978; Jokumsen and Fyhn, 1982; Booth *et al.*, 1984).

The effects of emersion on freshwater clams are known primarily from studies of the responses of the corbiculid bivalve, *Corbicula fluminea* (Müller) (McMahon, 1979, 1983; McMahon and Williams, 1984; Byrne *et al.*, 1988, 1989, 1990, 1991a, b). *C. fluminea,* however, is a relatively recent invader of freshwater (Keen and Casey, 1969) and so displays many responses thought to be intermediate between those of estuarine clams and those of more an-

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cient freshwater species (McMahon, 1979; Byrne *et al.*, 1988). Dietz (1974) described aerial O_2 consumption and responses to dehydration in the unionid, *Ligumia subrostrata*, and Heming *et al.* (1988) examined acid-base changes in extrapallial (mantle cavity) fluid of *Margaritifera margaritifera* during emersion. These studies indicate that freshwater bivalves possess adaptations to emersion that may account for some of the observed tolerances to aerial exposure. But the respiratory, acid-base, and ion regulatory consequences of emersion and subsequent reimmersion have not been comprehensively examined in a unionid clam.

Here we report an examination of such events during an extended bout of aerial exposure and for 24 h after reimmersion in the unionid bivalve *Anodonta grandis simpsoniana*. Although this species inhabits the shallow, littoral region of lakes, and members of the population under study could become aerially exposed with minor changes in lake level, such occurrences would be rare. Therefore, any compensations to aerial exposure and subsequent reimmersion that may be noted in this species may be interpreted as an inherent ability of unionid clams to withstand periods of emersion and not any particular response of an often exposed species.

Materials and Methods

Animals

Anodonta grandis simpsoniana is a northern nearctic species inhabiting the littoral region of boreal lakes. Specimens were collected at depths of between 1–7 m by SCUBA divers from Narrow Lake in north-central Alberta. Clams were returned to the laboratory within 6 h and maintained, unfed, at 10°C in aerated aquaria containing artificial pondwater (APW; composition in mmol l^{-1}: 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, 0.05 KCl; Dietz and Branton, 1975) for at least 2 weeks prior to experimentation.

Emersion and reimmersion

Seventy animals were numbered, and groups of 14 individuals were placed on plastic mesh platforms suspended 3 cm above the bottom in each of 5 covered plastic basins ($30 \times 25 \times 18$ cm). The clams were submerged to a depth of 2 cm above the animal with aerated APW at 10°C.

A preliminary experiment indicated that a 144 h (6 days) period of emersion resulted in a significant response without mortality. Thus, after a 24 h acclimation period in the containers, the clams were emersed by emptying the container to a level of 2 cm from the bottom thereby retaining a water reservoir to maintain the humidity in the basin near saturation. The water in the five basins was lowered at 10-min intervals so that the subsequent samples could be taken at the same nominal exposure time. After an emersion period, at constant temperature, of 144 h, the clams were reimmersed by gently filling the basins with temperature-equilibrated APW. The reimmersion period lasted 24 h. No mortality resulted during either the emersion or reimmersion periods.

At intervals of 0, 1, 7, 25, 48, 96, and 144 h emersion, and at 2, 6, and 24 h reimmersion, five clams (one per basin) were removed and an extracellular fluid (ECF) sample taken anaerobically by pericardiac puncture using cooled glass syringes (after the method of Fyhn and Costlow, 1975). ECF P_{O_2}, P_{CO_2} and pH were measured immediately on 500 μl samples using a Radiometer BMS 3 Mk 2 Blood Microsystem maintained at experimental temperature and connected to a Radiometer PHM 73 pH/Blood gas monitor. P_{O_2} and P_{CO_2} were determined by means of a Radiometer P_{O_2} (E5047-0) and P_{CO_2} (E5037-0) electrode respectively; pH was measured using a Radiometer glass capillary pH electrode (G299A). Total CO_2 (C_{CO_2}) was also measured immediately on 45 μl samples by means of a Corning 965 CO_2 Analyzer calibrated with standard $NaHCO_3$ solution. The remaining ECF (500–800 μl) was stored at 4°C for subsequent ion analyses. Immediately following sampling, the soft tissue of the clams was removed from the shell and dried to constant weight (>96 h) at 80°C.

ECF [Na] and [K] were measured using a Perkin-Elmer model 5000 atomic absorption spectrophotometer on samples diluted with 1% CsCl, and ECF [Ca] was determined on samples diluted with 1% $LaCl_3$. ECF [Cl] was measured by means of a Radiometer CMT10 chloride titrator.

In another experiment, 60 specimens were individually numbered, blotted of excess water, and weighed to the nearest 0.1 mg. Animals were placed in containers filled with APW at 10°C for 24 h, emersed for 144 h, and reimmersed for 24 h, as described previously. At the same time intervals as in the previous experiment, a sample of five animals was removed, blotted of excess water, and reweighed to determine water loss or gain. An ECF sample was drawn from each animal and its osmolality determined on 50 μl aliquots using a freezing point depression osmometer (Precision Systems μOsmette). The soft tissue was excised, dried (80°C, >96 h), and weighed; the shell was blotted dry and weighed. Total body water [total weight at the beginning of the experiment − (shell weight + dry tissue weight)] was calculated. All weight changes were assumed to be due to water loss or gain. The change in body water content was calculated from the weight change at the experimental time and was expressed as a percentage of the initial body water content.

In vitro determinations of CO_2 combining curves were performed on ECF drawn from the pericardial cavities of six animals. The sample from each animal was centrifuged at 6000 × *g*, and the supernatants from all samples were then pooled and maintained on ice. Aliquots (100 μl) of pooled ECF were placed in equilibration tubes and tonometered at 10°C in a Radiometer BMS2 Mk 2 Blood Micro System with $CO_2/O_2/N_2$ mixtures of 0.05%, 0.1%, 0.4%, 1.0%, 2.0% and 3.0% CO_2 in 50% O_2 and balance N_2 supplied by Wösthoff precision gas mixing pumps. After equilibration, ECF pH and C_{CO_2} were determined as described previously.

Calculations

The pK_{app} (apparent pK of the CO_2/bicarbonate buffer system) of clam ECF was calculated from the P_{CO_2} equilibration tension (Torr) and the corresponding measurements of pH and C_{CO_2} (mmol l^{-1}) for each *in vitro* sample. The calculation was carried out by rearrangement of the Henderson-Hasselbalch equation as follows:

$$pK_{app} = pH - \log\left(\frac{C_{CO_2}}{\alpha CO_2 \cdot P_{CO_2}} - 1\right)$$

The solubility coefficient of CO_2 (αCO_2) at 10°C was calculated by interpolation from the table in Cameron (1986) as 0.069 mmol (l Torr)$^{-1}$. As there was no relationship between pK_{app} and pH ($r = 0.01$; $P > 0.05$), the average value of pK_{app} (6.436 ± 0.035) was used to calculate the P_{CO_2} isopleths for the plot of pH against apparent bicarbonate concentration (see Fig. 6). The apparent bicarbonate (HCO_3app mmol l^{-1}) is that portion of the C_{CO_2} remaining after the dissolved CO_2 is removed; it is predominantly HCO_3, but includes carbonate, carbamates, and other ion pairs (see Heisler, 1986). Concentrations of apparent bicarbonate were calculated from C_{CO_2} according to the equation:

$$HCO_3app = C_{CO_2} - \alpha CO_2 \cdot P_{CO_2}$$

The HCO_3app and pH data were used to estimate the *in vitro* buffer capacity of the ECF as the change in HCO_3app per unit change in pH (Slykes). Apparent '*in vivo*' buffering capacity was calculated from measured HCO_3app and pH values over the first 96 h of emersion.

Net ion flux on reimmersion

Ten clams were aerially exposed for 6 days at 10°C under conditions of about 100% relative humidity. The clams were then placed in individual baths containing 50 ml APW. Once the clams had opened their valves and had commenced siphoning (5–15 min), bathing medium samples were taken; two additional samples were taken after 1 h and 2 h incubation. The clams were then returned to an aquarium containing aerated pondwater. At 6, 12, 24, 36, and 48 h reimmersion, the same clams were again placed in 50 ml APW and samples of bathing medium taken initially and 1 h later. Handling and other disturbances were minimized throughout. Undiluted samples were assayed for Na and K by emission spectroscopy with a Perkin-Elmer model 5000 atomic absorption spectrophotometer. Calcium concentration was determined by atomic absorption spectroscopy on samples diluted with $LaCl_3$, while Cl content was measured by coulometric titration on a Radiometer CMT10 adjusted to assay low concentrations (± 0.05 mmol · l^{-1}). The soft tissues of each clam were excised and dried to constant weight at 80°C (96 h). The net ion flux (J_{net}) during each sampling period was calculated from the change in the ionic content of the bathing medium over time, and normalized to the dry weight of tissue. The net ion fluxes of a control group of ten continually immersed clams were also determined.

Statistics

All data are expressed as means ± SEM. For the changes in ECF ion and acid-base variables, a single classification ANOVA was performed with time of sample as the classificatory variable; emersion and reimmersion samples were both included. If the ANOVA proved to be significant ($P < 0.05$), sequential contrasts were performed in which the control value was compared with each experimental value to detect specific significant differences ($P < 0.05$). SYSTAT procedures were used for statistical analyses. The relationship between [Ca] and [HCO_3app] was tested by least squares linear regression. For the net ion flux experiment, a repeated measures ANOVA was performed on the flux of each ion over time. The mean fluxes were compared to the flux of the control group, and the difference and its significance were determined by post-hoc Neuman-Keul's tests ($\alpha = 0.05$) using the repeated measures ANOVA mean square error term and an "n" of 10.

Results

All experimental specimens of *Anodonta grandis simpsoniana* survived 6 days of aerial exposure at 10°C and a subsequent 24 h of reimmersion. While emersed, the clams lost about 1.6% of their total body water per day (Fig. 1). The weight loss was consistently significantly greater than pre-emersion controls by 48 h emersion. ECF osmolality increased from a control value of 42 ± 1 mOsm kg^{-1} to 54 ± 2 mOsm kg^{-1} by 144 h emersion (Fig. 1). This constituted a 28% increase in solute whereas water loss was only 13%. ECF osmolality was significantly related to emersion time:

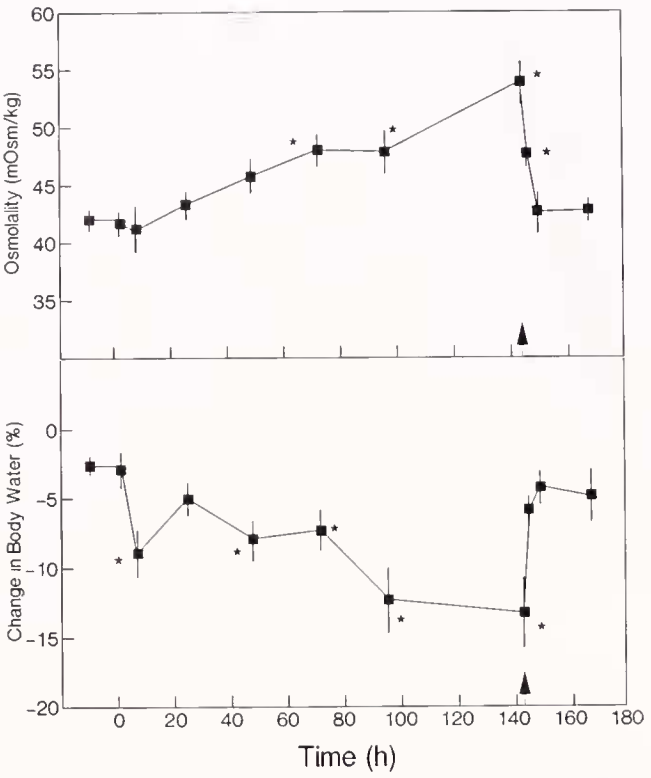

Figure 1. Time course of changes in ECF osmolality (upper panel) and body water content (lower panel) in *Anodonta grandis simpsoniana* during 144 h emersion and 24 h reimmersion in pondwater at 10°C. Symbols are mean values for five clams; bars are standard errors of the mean. Asterisks indicate values significantly different from control ($P < 0.05$). The arrows indicate the time of reimmersion in pondwater.

$$\text{Osmolality} = 41.4\ (\pm 3.2) + 0.082\ (\pm 0.010) \times \text{emersion time (h)}$$

$$(\pm\text{SE};\ R^2 = 0.63;\ \text{df} = 1, 38)$$

This translates to an increase in osmolality of approximately 2 mOsm kg^{-1} d^{-1}. Reimmersion resulted in return of water weight to control within 2 h. ECF osmolality declined to control within 6 h of resubmergence.

During the first hour of emersion the clams displayed a significant decline in ECF P_{O_2}; thereafter, a further, but slower decrease occurred over the course of the emersion period (Fig. 2). Values remained above 20 Torr throughout the time that the animals were exposed. During reimmersion, the ECF was rapidly reoxygenated, but not to pre-emersion controls values, even after 24 h of resubmergence.

ECF P_{CO_2} rose steadily during the first 24 h of emersion, reaching 7.7 ± 1.1 Torr (Fig. 2). Thereafter, P_{CO_2} continued to rise, but at a slower rate, and after 96 h of emersion values stabilized near 13 Torr. On reimmersion, ECF P_{CO_2} declined to pre-emersion values within 6 h of resubmergence.

Emersion resulted in an ECF acidosis, with pH falling significantly below pre-emersion values after the clams had been in air for 7 h (Fig. 2). Although the acidosis was progressive, the rate of pH decline slowed after 24 h and reached a seemingly stable level near 7.5; there it remained until the end of the emersion period. But the steady pH is misleading, as $[H^+]$ continued to rise until 96 h after the onset of emersion; then it remained unchanged. ECF pH returned to control values within 2 h of resubmergence.

Both ECF [Ca] and $[HCO_3app]$ changed significantly with the duration of exposure and on return to water (Fig. 3). ECF $[HCO_3app]$ increased rapidly to 8.8 ± 0.6 mmol l^{-1} during the first 24 h of exposure and then rose less rapidly to 12.9 ± 0.8 mmol l^{-1} by the end of the emersion period. ECF [Ca] had almost doubled from 5.5 ± 0.4 mmol · l^{-1} to 10.0 ± 0.5 mmol · l^{-1} after 48 h emersion and continued to rise, but at a slower rate, reaching 13.1 ± 0.8 mmol · l^{-1} after 144 h in air. When the clams were reimmersed, the ECF concentrations of both Ca and HCO_3app declined rapidly. [Ca] returned to pre-emersion

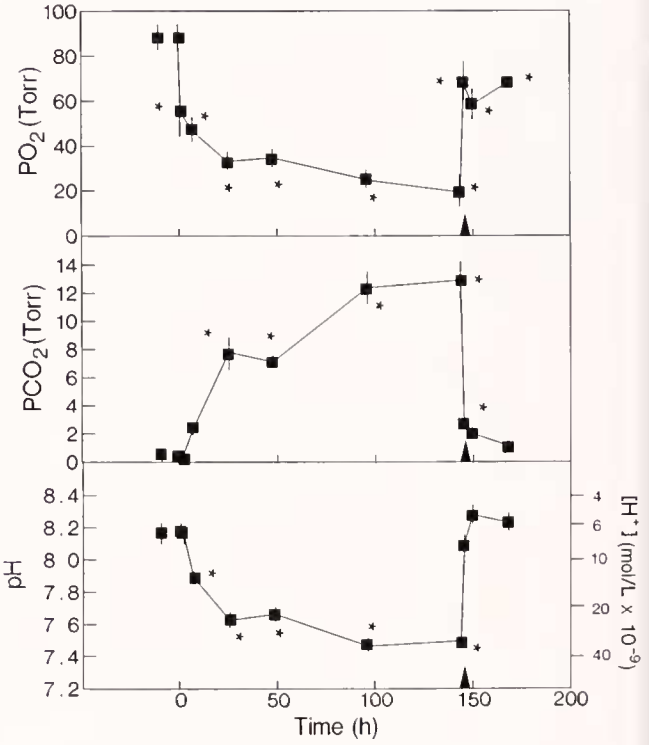

Figure 2. Time course of changes in ECF P_{O_2} (upper), P_{CO_2} (middle), and pH (lower) in *Anodonta grandis simpsoniana* during 144 h emersion and 24 h reimmersion in pondwater at 10°C. The control condition (time = 0) is repeated to the left for clarity. Symbols are mean values for five clams; bars are standard errors of the mean. Asterisks indicate values significantly different from control ($P < 0.05$). The arrows indicate the time of reimmersion in pondwater.

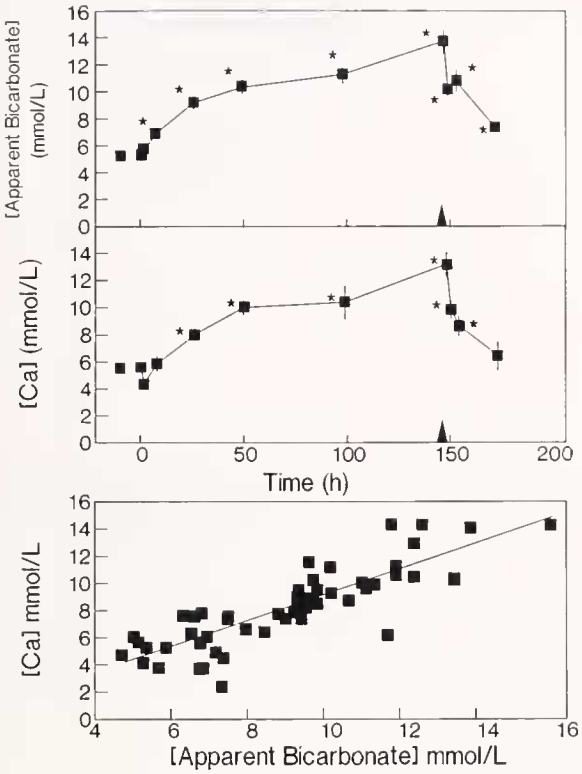

Figure 3. The upper two panels show the time course of changes in extracellular [HCO_3app] (upper), and [Ca] (middle) in *Anodonta grandis simpsoniana* during 144 h emersion and 24 h reimmersion in pondwater at 10°C. The control condition (time = 0) is repeated to the left for clarity. Symbols are mean values for five clams; bars are standard errors of the mean. Asterisks indicate values significantly different from control ($P < 0.05$). The arrows indicate the time of reimmersion in pondwater.

The lower panel shows the relationship between [Ca] and [HCO_3app] in the extracellular fluid of both emersed and reimmersed specimens of *Anodonta grandis simpsoniana*. Note the 1:1 stoichiometry between these two ion species.

values by 24 h resubmergence whereas, after the same period of reimmersion, HCO_3app remained elevated over controls.

The pattern of increase and decline in ECF Ca and HCO_3app was strikingly similar. Indeed, there was a 1:1 stoichiometric relationship between ECF levels of Ca and HCO_3app (Fig. 3) measured throughout the emersion and reimmersion periods. The regression equation relating [Ca] to [HCO_3app] was:

$$[Ca] = -0.6 + 0.99*[HCO_3app]$$

$$(R^2 = 0.72; df = 1, 48)$$

The two other major ECF ions, Na and Cl, did not change significantly throughout either the emersion or reimmersion periods (Fig. 4). The slight decrease in concentrations of both these ions on reimmersion was not significant. ECF [K] constituted a small proportion of the total ionic strength but showed significant increases between 24 and 48 h emersion, and then declined to control levels for the balance of the emersion period and throughout the reimmersion period.

As the protein concentration in clam ECF is low (0.24 ± 0.03 $g \cdot l^{-1}$; R. A. Byrne and B. R. McMahon, unpub. data), the *in vitro* non-bicarbonate buffering capacity of the ECF was also low. The *in vitro* relationship between pH and HCO_3app was:

$$[HCO_3app] = 13.64\ (\pm 0.30) - 0.68\ (\pm 0.20)*pH$$

$$(R^2 = 0.75, df = 1, 4).$$

During the first 96 h of emersion, the relationship between ECF pH and apparent bicarbonate described a steeper linear association. This *in vivo* relationship yielded the following regression equation:

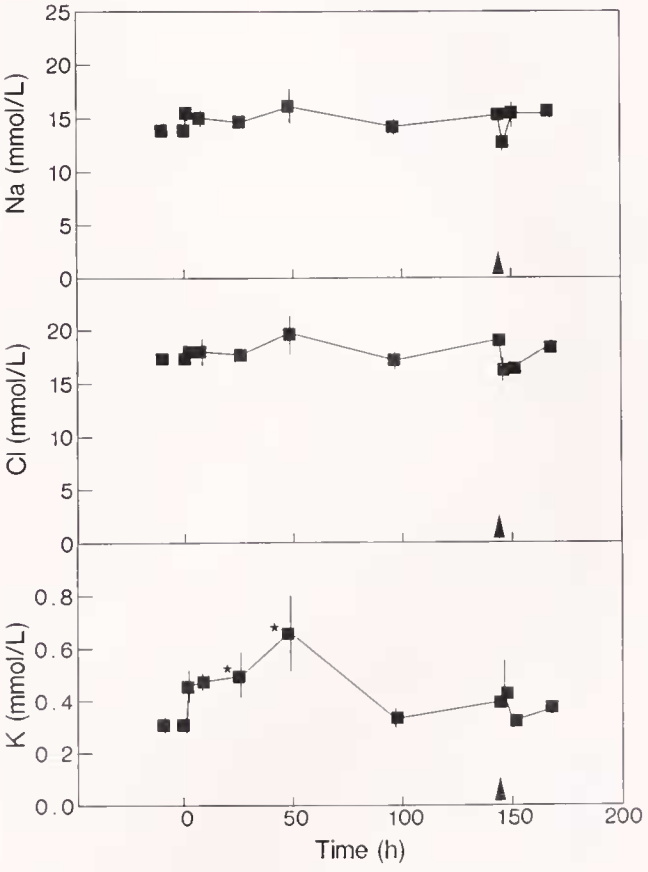

Figure 4. Time course of changes in extracellular [Na] (upper panel), [Cl] (middle panel), and [K] (lower panel) in *Anodonta grandis simpsoniana* during 144 h emersion and 24 h reimmersion in pondwater at 10°C. The control condition (time = 0) is repeated to the left for clarity. Symbols are mean values for five clams; bars are standard errors of the mean. Asterisks indicate values significantly different from control ($P < 0.05$). The arrows indicate the time of reimmersion in pondwater.

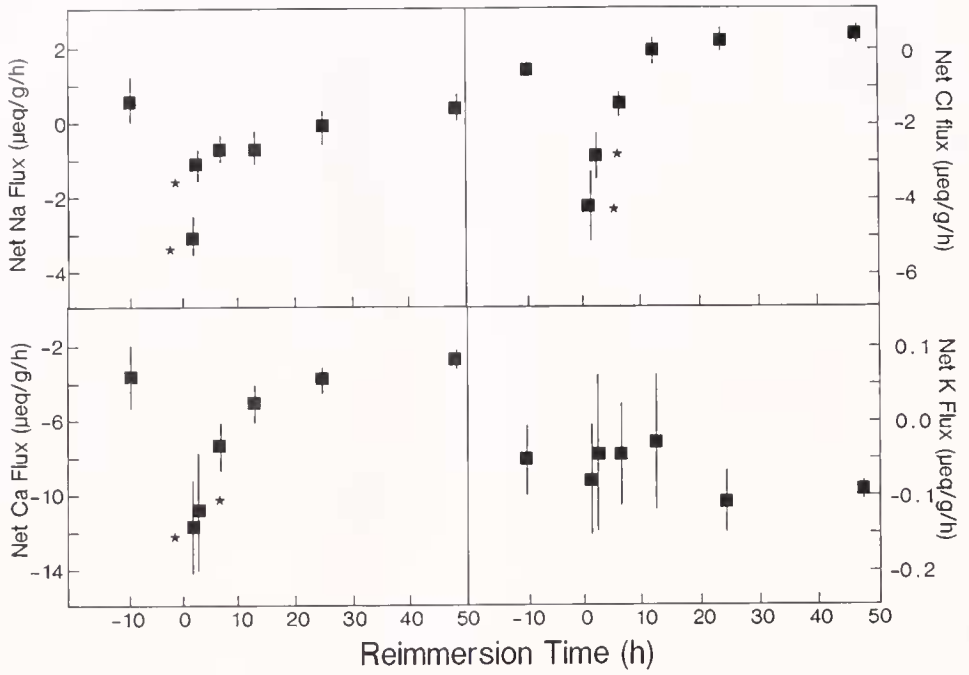

Figure 5. The time course of net ion flux ($\mu eq \cdot g^{-1} \cdot h^{-1}$) for specimens of *Anodonta grandis simpsoniana* over a 48 h reimmersion period following 144 h emersion at 10°C. Values are for Na, Cl, Ca, and K. Asterisks indicate significant differences when compared to the pre-emersion value ($P < 0.05$). Symbols are means of values for ten animals; bars are standard errors of the mean.

$$[HCO_3app] = 56.80\ (\pm 1.32) - 6.24\ (\pm 0.87) * pH$$

$$(R^2 = 0.65,\ df = 1, 28),$$

indicating an "*in vivo*" buffering capacity almost 10 fold higher than that suggested by the *in vitro* determination.

When clams were reimmersed after 6 days of aerial exposure, there were immediate and large losses of calcium, sodium, and chloride from the animals (Fig. 5), followed by a return to control flux levels over the reimmersion period. Initial (first hour) loss of calcium was the greatest, at $-11.7 \pm 2.5\ \mu eq \cdot g^{-1} \cdot h^{-1}$, whereas the value for sodium was -3.0 ± 0.5, and for chloride $-4.3 \pm 0.9\ \mu eq \cdot g^{-1} \cdot h^{-1}$. There was no significant change in potassium flux over the reimmersion period, but there was a large variation in initial flux (Fig. 6). Net fluxes for sodium, calcium, and chloride were not significantly different from control fluxes by 6 h reimmersion. Fluxes for sodium and chloride attained steady state ($J_{net} \sim 0$), whereas calcium net fluxes were always negative.

Discussion

Changes in ECF acid-base status during emersion and on reimmersion in *Anodonta grandis simpsoniana* are summarized in the pH-apparent bicarbonate diagram (Fig. 6). The clams experienced a respiratory acidosis during emersion that was partially compensated by increases in apparent bicarbonate concentration. After 96 h of emersion, the pH stabilized, and further increases in HCO_3app were not associated with an increase in P_{CO_2}. On reimmersion, a rapid rise in pH was accompanied by a fall in P_{CO_2} and bicarbonate. Although pH and P_{CO_2} returned to control levels rapidly, bicarbonate concentrations remained elevated even after 24 h reimmersion.

This pattern of acid-base response to emersion is similar to that reported for the freshwater corbiculid bivalve, *Corbicula fluminea* (Byrne *et al.*, 1991b) in that the acidosis is progressive, and the rise in HCO_3app keeps pace with the development of the respiratory component of the acidosis. The littoral crab *Pachygrapsus crassipes* also shows a qualitatively similar pattern when emersed (Burnett and McMahon, 1987).

Valve gaping, a behavior in which the shell valves open periodically and which may allow limited gas exchange, was observed in emersed *A. grandis simpsoniana*. ECF P_{O_2} never went below 20 Torr and P_{CO_2} stabilized at near 13 Torr, suggesting that clams were either not completely closed systems, *i.e.*, that O_2 and CO_2 could diffuse in and out, respectively, or that a reduction in aerobic metabolism had taken place. The 1:1 stoichiometry between calcium and apparent bicarbonate suggests an equilibrium resulting from

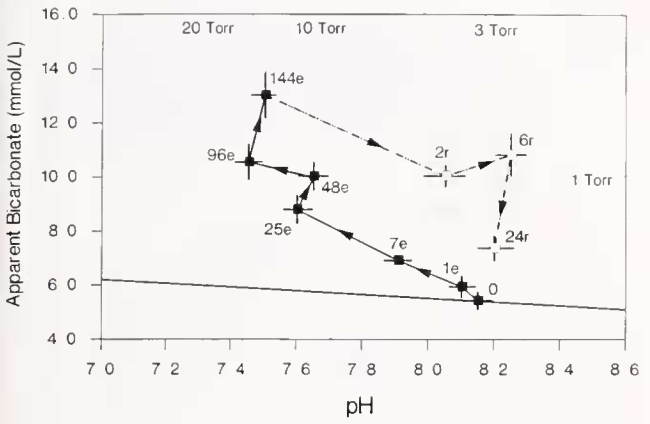

Figure 6. Diagram summarizing changes in extracellular pH, apparent bicarbonate and P_{CO_2} in *Anodonta grandis simpsoniana* during 144 h emersion and 24 h reimmersion in pondwater at 10°C. Closed symbols joined by solid lines are values for emersed clams while stippled symbols connected by dashed line are those of reimmersed clams. Values are means and vertical and horizontal bars are SEM's for HCO_3app and pH, respectively. The arrows indicate the time course while the numbers alongside the symbols indicate the length of time, in hours, emersed (e) or reimmersed (r). The solid straight line represents the *in vitro* non-bicarbonate buffer line for clam ECF at 10°C.

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3^-$$

In a completely closed system, the levels of HCO_3^- and Ca would rise as long as protons were being produced. In the emersed, incompletely closed clam, the levels of HCO_3^- that are accumulated are determined by the ability of the clam to offload CO_2 to the environment. The attainment of the new equilibrium between CO_2, HCO_3^-, and Ca^{2+} is achieved after a period in air, during which ECF P_{CO_2} rises to the "equilibrium" level. In *Anodonta grandis simpsoniana* at 10°C, this process takes 96 h. The continued production of Ca and HCO_3^- after this time suggests that the buffering of protons continues with the further dissolution of $CaCO_3$, but that an equilibrium has been established with CO_2 being released.

The effectiveness of the shell buffer system, presumably the major buffer system in operation, can be seen by the 10 fold increase in buffering capacity of the "*in vivo*" system over the *in vitro* determinations on isolated ECF. Mobilization of $CaCO_3$ from shell has been implicated in acid-base control in a number of bivalve species. During aerial exposure, calcium levels in the body fluids of *Mya arenaria* rose (Collip, 1920). Similarly, when the marine bivalve *Mercenaria mercenaria* was emersed, CO_2 and calcium levels in the ECF increased (Dugal, 1939). The source of calcium is from the shell as was reported by Crenshaw and Neff (1969) using ^{45}Ca labelling techniques. Later, it was shown that calcium appears in the extrapallial fluid before entering the extracellular compartment (Crenshaw, 1972), confirming that shell dissolution is the cause of increased calcium. A cyclical change in ECF calcium, presumably associated with HCO_3app production, was noted by Akberali *et al.* (1977). When the clam had closed valves and became hypoxic, calcium levels rose; during bouts of valve opening when ventilation would recommence, calcium levels declined. Additionally, Booth *et al.*, (1984) did not find any change in either ECF pH or [Ca] in *Mytilus edulis* during short-term emersion during which metabolism remained predominantly aerobic. However, under conditions of extended (6 days) aerial exposure, P_{CO_2} in the ECF of *Mytilus edulis* and *Modiolus modiolus* rose along with a decrease in pH (Jokumsen and Fyhn, 1982).

The very limited non-bicarbonate buffer system is balanced by the large store of readily mobilizable shell buffer. This is especially important for unionid clams, which have evolved a very dilute body fluid as an adaptation to life in freshwater. The low ionic strength of both the extracellular and intracellular compartments reduces the energy required to maintain homeostatic ionic levels by reducing the diffusive gradient. Nevertheless, emersion resulted in a large increase in extracellular solute. ECF osmolality of *Corbicula fluminea* during three days' aerial exposure at 25°C increased twofold (Byrne *et al.*, 1989), and a similar increase was noted for the unionid, *Ligumia subrostrata*, during a 5-day emersion period (Dietz, 1974). However, death from aerial exposure does not seem to result solely from a simple lethal increase in solute, as *C. fluminea* succumbed to aerial exposure after losing total body water ranging from 30 to 80% (Byrne *et al.*, 1988). Rather, death seems related to the inability of the clams to maintain acid-base balance or to avoid the production of toxic end-products (Byrne *et al.*, 1991a, b).

Extracellular sodium and chloride remain highly regulated both during emersion and during the reimmersion period. A similar situation was reported for *C. fluminea* during aerial exposure and subsequent resubmergence (Byrne *et al.*, 1989). Reports of changes in ECF contents of these ions in other bivalves undergoing emersion generally show a gradual increase in ECF ion concentrations related to the duration of aerial exposure. *Ligumia subrostrata* had an increased level of extracellular sodium and chloride proportional to the level of desiccation stress upon aerial exposure (Dietz, 1974). Similarly, chloride levels increased passively as body fluids became more concentrated during emersion in *Mytilus edulis* and *Modiolus modiolus* (Jokumsen and Fyhn, 1982). In severe hypoxic exposure, however, ECF sodium levels in *Scrobicularia plana* remained constant over time (Akberali *et al.*, 1977). The constant extracellular fluid [Na] and [Cl] in emersed *Anodonta grandis simpsoniana* suggests that these ions are moving into some other compartment, *e.g.*,

intracellular space. This possibility is further evinced by the losses of these ions on reimmersion, even though ECF levels are not significantly altered. It has been postulated that, in *Corbicula fluminea,* these ions are shunted to the intracellular space to conserve cell volume (Byrne *et al.,* 1989), and a similar situation may be occurring in *A. grandis simpsoniana.* In any case, the tight regulation of these ions suggests that maintaining constant extracellular concentrations of sodium and chloride is a fundamental requirement and may be associated with maintenance of electrochemical balance.

On reimmersion, the accumulated extracellular calcium is removed over a 24 hour period, and ECF calcium levels return to control values. The loss is also evident by the high negative net fluxes for this ion during the initial stages of reimmersion. Other unionid clams recapture extracellular calcium in concretions made up primarily of calcium phosphate during and after periods of hypoxia (Silverman *et al.,* 1983). Although *A. grandis simpsoniana* possesses concretions (Byrne, McMahon, Silverman, and Dietz; unpubl. data), the capacity for extracellular calcium recapture is not known. As P_{CO_2} falls to control levels almost immediately on reimmersion, a sustained high calcium level in the ECF would result in an imbalance in the strong ion difference which would lead to an alkalosis. The loss of calcium on reimmersion (amounting to over 50 μeq for a 1 g dry weight animal in the first 6 h of reimmersion), however, must be regained at a later time, either from the diet or by epithelial transport mechanisms.

The lower ECF P_{O_2} on reimmersion perhaps was due to an enhanced ventilation and oxygen consumption, as valve gape seemed to be increased and ECF P_{CO_2} declined rapidly. This response is seen in intertidal bivalves, even those that remain fully aerobic during aerial exposure (Widdows *et al.,* 1979; Widdows and Shick, 1985).

In general, freshwater bivalves are more tolerant of extended periods of emersion than are estuarine or intertidal species (McMahon, 1979; Byrne *et al.,* 1988). As the freshwater bivalve, *Corbicula fluminea,* displayed intermediate responses to emersion and had the lowest reported tolerance to emersion for a freshwater species, it was thought that the greater capacity to survive aerial exposure of unionid species might be due to a more complete compensation for the consequences of emersion, in particular maintenance of acid-base and ion balance (Byrne *et al.,* 1991b). However, *Anodonta grandis simpsoniana* displays responses to emersion that seem to be similar to those of *C. fluminea.* The use of shell buffer to combat a predominantly respiratory acidosis; release of accumulated CO_2 and aerial O_2 consumption while minimizing water loss; redistribution of extracellular sodium and chloride; rapid recovery when reimmersed brought about by increased ventilation; and a loss of a large amount of ions may thus be general adaptations of freshwater bivalve species. Therefore, the extended tolerance of aerial exposure displayed by many unionid species may not be due to behavioral or physiological adaptations particularly unique to the group, but may simply reflect a wide latitude of capacity adaptation.

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